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Toxicokinetics of Polybrominated Diphenyl Ether Congeners 47, 99, 100, and 153 in Mice

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The congener profiles of polybrominated diphenyl ethers (PBDEs) in human and wildlife samples are dominated by brominated diphenyl ether (BDE) congeners 47, 99, 100, 153, and 154, all of which are components of the commercial pentaBDE mixtures commonly used in a variety of flammable consumer products. Very little information is available on the toxicokinetics of these congeners and no studies are available directly comparing these BDE congeners in mice. Therefore, the objective of this study was to compare the distribution, metabolism, and excretion of BDEs 47, 99, 100 and 153. Female C57BL/6 mice were administered a single dose of BDE (1 mg/kg: 2.1, 1.9, 1.9, and 1.8 μmol/kg, respectively) intravenously. Excretion was monitored daily, and terminal tissue disposition was examined 5 days following exposure. All BDE congeners in this study distribute with similar patterns into lipophilic tissues; however, tissue concentrations 5 days following exposure were much higher for BDE-153 than for 100, 99, and 47, respectively. Excretion rates were inversely related to tissue concentrations as BDE-47 was the most rapidly excreted congener, followed by BDE-99, -100, and -153. Differences in tissue concentrations were largely driven by congener-specific urinary elimination rates which were associated with protein binding in the urine. While the overall rate of metabolism appeared to be low, analysis of metabolites in daily feces samples revealed that BDE-99 was the most rapidly metabolized congener in this study. The results of this study demonstrate that congener substitution plays a role in the distribution, metabolism, and excretion of PBDEs in mice and it is therefore important to consider the differential toxicokinetic parameters associated with each congener when assessing the risk to human health from these PBDE congeners.

Key Words: **brominated flame retardant (BFR); toxicokinetics; polybrominated diphenyl ether (PBDE).**

Polybrominated diphenyl ethers (PBDEs) are commercial chemical products used to prevent combustion of various consumer goods. Recently, concern for PBDEs has risen due to their detection in the environment and in human biota (Birnbaum and Staskal, 2004). There are three major commercial mixtures of PBDEs: decabromodiphenyl ether (DecaBDE; deca); octabromodiphenyl ether (OctaBDE; octa); and pentabromodiphenyl ether (PentaBDE; penta). The PBDEs potentially involve 209 different congeners, varying in both number and position of bromines on the diphenyl ether structure. Commercial octa and deca are primarily used as additive flame retardants in high-impact polystyrene, textiles, polyethylene, and epoxy resins. Final consumer applications include, but are not limited to, electrical and electronic equipment, circuit boards, cellular phones, sofas, office chairs, and many types of reinforced plastics used in construction and transportation (BSEF, 2004).

The third commercial PBDE product, PentaBDE, is a viscous liquid used primarily as an additive in flexible polyurethane foams, where up to 30% of the weight of the foam can be accounted for by this flame retardant (Hale *et al.*, 2002). PentaBDE has been used as a flame retardant in PUF since the 1970's in consumer goods such as carpet padding, sofas, and mattresses. PentaBDE also has minor uses in phenolic resins, polyesters, and epoxy resins. Although there is variation in commercial mixtures, penta products are generally composed of 24–38% tetrabromodiphenyl, 50–60% pentabromodiphenyl, and 4–8% hexabromodiphenyl ethers.

The commercial pentaBDE mixture, as well as individual congeners, has been shown to cause developmental neurotoxicity and reproductive toxicity, and the potential to disrupt endocrine function in rodent studies. Results of Stoker *et al.* (2004, 2005) have demonstrated that DE-71, a commercial penta mixture, can impair reproductive development when administered as part of an endocrine disruptor screening protocol in rats. These studies have also evaluated the reproductive antiandrogenic properties of DE-71 both *in vivo* and *in vitro*. Several studies have also shown that DE-71 and the individual congeners alter thyroid hormone status (Hakk *et al.*, 2002; Hallgren *et al.*, 2001; Zhou *et al.*, 2002). However, much

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of the public health concern for the potential human health effects of PBDEs comes from the reports of developmental neurotoxicity. Mice exposed to BDEs 47, 99, 153, and 209 on postnatal day 10 showed neurobehavioral alterations at doses as low as 0.4 mg/kg (Eriksson *et al.*, 2001, 2002; Viberg *et al.*, 2002, 2003, 2004a,b). Furthermore, the results of these studies show that the deficits in learning and memory are observed in adulthood and the effects worsen with age. Other reports have also shown neurotoxicity following neonatal exposure (Branchi *et al.*, 2002; Ceccatelli *et al.*, 2006; Lilienthal *et al.*, 2006).

A common theme emerges when evaluating exposure to PBDEs: brominated diphenyl ether (BDE) 47 is the major PBDE congener found in most human and environmental biota, followed by BDEs 99, 100, 153 and 154 (Hites, 2004). These five congeners are the main components of the pentaBDE commercial mixtures which have primarily been used in the United States; however, they have also consistently been the dominant congeners found worldwide regardless of the use of pentaBDE in regional consumer products (Birnbaum and Staskal, 2004). Whether this phenomenon is a product of debromination of more highly brominated PBDEs, differential metabolism or other pharmacokinetic issues, or reflects their greater environmental persistence is not yet clear.

The need to understand the kinetics of the congeners predominantly measured in humans and the environment is an essential component to assessing the risk of these flame retardant chemicals. To our knowledge, there are currently no studies available which directly compare the toxicokinetics of multiple BDE congeners. Several studies have evaluated BDE-47 kinetics following single doses (Darnerud and Risberg, 2006; Orn and Klasson-Wehler, 1998; Sanders *et al.*, 2006; Staskal *et al.*, 2005, 2006a) and demonstrate that BDE-47 distributes to lipophilic compartments, is not readily metabolized, and exhibits a species difference between rats and mice with respect to urinary and fecal excretion profiles. In mice, urinary excretion is a major route of elimination whereas it plays a very minor role in rats.

Hakk *et al.* (2002) evaluated the toxicokinetics of BDE-99 in conventional and bile duct-cannulated male rats. Results indicated that lipophilic tissues were preferred sites for disposition, urinary excretion was very low, and the majority of the dose was excreted in the feces as unmetabolized parent compound. Chen *et al.* (2006) has also investigated the toxicokinetics of BDE-99 in both rats and mice. While the results generally agreed with the Hakk *et al.* (2002) study, Chen *et al.* (2006) also demonstrated that BDE-99 accumulated in tissues following repeated dosing in rats. Hakk *et al.* (2006) has recently published results of a toxicokinetic study in rats with BDE-100 in which lipophilic tissues were the preferred sites for disposition. Urine and biliary excretion were very low (< 1% of dose) and almost 20% of the dose was measured in feces mainly as the unmetabolized parent in bile cannulated rats, and > 70% of the radiolabeled oral dose was retained at 72 h following exposure in the conventional rats. To our knowledge, toxicokinetic studies for BDE-153 have not been published.

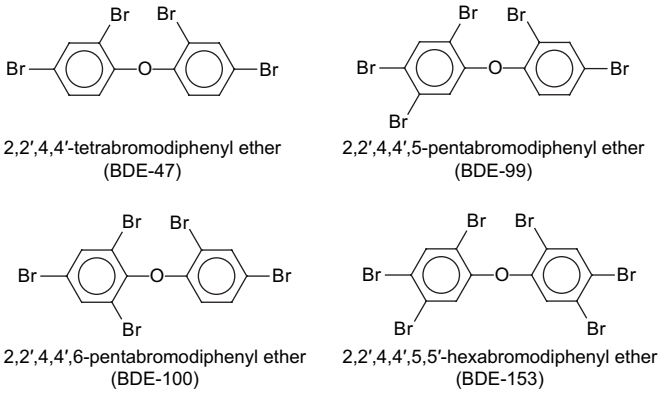


FIG. 1. Structure of BDEs 47, 99, 100, and 153.

While the library of toxicokinetic literature on PBDEs is growing, the available studies indicate significant differences between congeners and between species. Therefore, this study was designed to investigate the toxicokinetics of BDEs 47, 99, 100 and 153 (Fig. 1) in mice. The results of this study present distribution, metabolism, and excretion parameters in female C57BL/6 mice following a single intravenous dose of [^{14}C] BDE-47, -99, -100, or -153 (1 mg/kg; 2.1, 1.9, 1.9, and 1.8 μmol/kg, respectively).

MATERIALS AND METHODS

Chemicals. Uniformly labeled [^{14}C] 2,2′,4,4′-tetraBDE (BDE-47) was generously provided by Great Lakes Chemical Corporation (Indianapolis, IN). [^{14}C] 2,2′,4,4′,5-pentaBDE (BDE-99) and [^{14}C] 2′,4,4′,5,5′-hexaBDE (BDE-153) was kindly provided by Dr Tom Burka National Institute of Environmental Health Sciences, and [^{14}C] 2,2′,4,4′,6-pentaBDE (BDE-100) was synthesized by Dr Janice Huwe (United States Department of Agriculture Agricultural Research Service–Biosciences Research lab, Hakk *et al.*, 2006). Chemical purity (> 97%) was determined by reverse-phase high-pressure liquid chromatography (System Gold, Beckman Instruments, Inc., Fullerton, CA) using an Ultrasphere ODS column (5 μm, 25 × 4.6 cm, Beckman Instruments, Inc.) and a gradient elution of 50:50 methanol:water over 30 min to 100% methanol at a flow rate of 1.5 ml/min. A radiometric flow detector (Beckman Model 171, Beckman Instruments), used with 1 ml/min Flo Scint III (Packard Instrument Co., Meridian, CT) was used to monitor radiopurity. All other chemicals used were of the highest grade commercially available. Diazomethane was synthesized in-house from Diazald (Sigma-Aldrich, St Louis, MO) according to the manufacturer's instructions.

Animals. All animal procedures were approved by the United States Environmental Protection Agency (USEPA) National Health and Environmental Effects Research Laboratory Institutional Animal Care and Use Committee. Female C57BL/6 mice were obtained from Charles River Laboratory (Raleigh, NC). Female mice were used in this study in order to compare present results to those of previous toxicokinetic studies in our laboratory as well as to compliment studies by other investigators, who have investigated similar endpoints in male rodents. Animals were maintained on a 12-h light/dark cycle at ambient temperature (22°C) and relative humidity (55 ± 5%), and were provided with Purina 5001 Rodent Chow (Ralston Purina Co., St Louis, MO) and tap water *ad libitum*. Prior to commencement of the study, mice were

adapted (three mice per cage) for 1 week in Nalge metabolism cages (Nalgene, Rochester, NY). Following administration of the BDE congener, mice were housed individually. All mice were 10 weeks of age and weighed approximately 20 g at time of treatment.

Dosing solution. A stock solution of [^{14}C]BDE-47 was made by sonicating 63.6 mg of [^{14}C]BDE-47 (55 μCi/mg) in toluene (1 ml) until dissolved. Aliquots from this stock were used to prepare the BDE-47 dosing solution. [^{14}C]BDE-99, [^{14}C]BDE-100, and [^{14}C]BDE-153 were dissolved in acetone (35.6, 0.21, and 27.8 mCi/mmol). Intravenous dosing solution was prepared in an Alkamuls EL-620 (formerly Emulphor: Rhone-Pouleax, NJ), ethanol, water (1:1:8) vehicle. Solvent from labeled congeners was allowed to air dry in an amber vial. When the BDE congener was approximately 80% dry, Alkamuls EL-620 was added, sonicated for 1 h, followed by organic solvent evaporation using a speed vacuum. Ethanol and water were then added. All solutions were made 24 h prior to administration and sonicated 2 h prior to administration. For each congener, animals received the following approximate amounts of radioactivity: [^{14}C] BDE-47 (1.2 μCi), [^{14}C] BDE-99 (1.5 μCi), [^{14}C] BDE-100 (0.08 μCi), or [^{14}C] BDE-153 (1.0 μCi).

Treatment. Mice were administered a single dose of BDEs 47, 99, 100, or 153 (1 mg/kg) intravenously via the tail vein at a dosing volume of 4 ml/kg (n = 4–8 animals per congener). Mice were housed individually in metabolism cages following BDE administration for 5 days. Urine and feces were collected daily. Five days following exposure, mice were euthanized by CO_2 asphyxiation followed by exsanguination via cardiac puncture. Blood, liver, lung, kidneys, skin, tail, adipose (abdominal), muscle (abdominal), and brain were collected and analyzed for residual radioactivity.

Tissue distribution. Residual radioactivity in the tissues was determined by combustion (Packard Oximate 307 Sample Oxidizer/Oximate 80 Operator, Downers Grove, IL) of triplicate samples when available (~100 mg per sample) followed by liquid scintillation spectrometry (LSS) of generated [^{14}C]CO_2 (Beckman LS6000LL Scintillation Counter, Beckman Instruments). Feces were air dried following collection, weighed, and ground. Triplicate samples were analyzed for radioactivity by combustion and LSS. Daily urine volumes were recorded, and 100 μl aliquots (triplicate) were analyzed by direct addition into scintillant for radioactivity determination by LSS. Remaining urine was then treated with a 2% sodium azide (Sigma S-8032) solution prepared in phosphate-buffered saline (Sigma P-4417) with a pH of 7.4 and stored at 4°C. Treated urine and fecal samples were transferred to the USDA for metabolite determination.

Urinary metabolites. Zero to 24 h urine from BDE-47 and -99 dosed mice, and because of limited amounts of radioactivity, 0–120 h urine from BDE-100 and -153 dosed mice were chromatographed by size exclusion on Sephadex G-75 (G-75; 4.5 × 90 cm; Pharmacia Biotech, Piscataway, NJ), as described previously (Larsen *et al.*, 1990a). The columns were eluted with 0.05M potassium phosphate buffer (pH 7.2). Elution fractions (20 ml) were assayed for radioactivity. Protein-bound fractions were assayed for protein by the Bradford method (bovine serum albumin as protein standard). Refined gel permeation chromatography (GPC) was performed with protein-bound fractions on a Sephacryl S-200 column (S-200; 2.2 × 85 cm; Pharmacia Biotech). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 13% acrylamide) was performed as described previously (Mazel, 1971) on protein-bound fractions. All electrophoresis materials were purchased from Bio-Rad Laboratories (Richmond, CA). The protein-bound fractions from urine were extracted with hexane and ethyl acetate (3× each). The combined organic layers were concentrated under a stream of nitrogen, and spotted onto silica gel thin-layer chromatography (TLC) plates (5 × 20 cm; 250 μm thickness; Analtech, Newark, DE). TLC plates were developed in 1:1 hexane:methylene chloride with a standard lane containing [^{14}C] parent compound. Ethyl acetate extracts of unbound urine from G-75 columns were applied to silica gel TLC plates and developed as described above.

Fecal metabolites. Feces were fractionated by the method of Orn and Klasson-Wehler (1998). Briefly, feces (on a daily and congener-specific basis) were weighed then extracted in a Soxhlet apparatus with 200 ml of 2:1 chloroform:methanol (Extract 1). The Soxhlet residue was further extracted with 200 ml of methanol (Extract 2). Extract 1 was evaporated, then partitioned between hexane (organic phase) and 0.1M H_3PO_4 (aqueous phase). The organic phase of Extract 1 was subjected to GPC partitioning (Bio Rad Bio-Beads SX-3 resin; eluted with 1:1 hexane:methylene chloride at 2 ml/min) to yield a lipid-bound fraction (LF) and free metabolite fraction (MF). The aqueous phase of Extract 1 was combined with Extract 2 to yield a water-soluble MF. Residue from the Soxhlet was combusted in a Model 307 tissue oxidizer (Packard, Meridan, CT) to yield a nonextractable fraction. Metabolites were characterized in the MF by the method of Bergman *et al.* (1994). Briefly, the MF was diluted with one volume of water and one volume of methanol. The samples were acidified with sulfuric acid (0.5M) and then extracted with hexane:methyl *tert*-butyl ether three times. The combined organic phases were concentrated and the lipid content determined gravimetrically. The extracts were redissolved in hexane and partitioned with potassium hydroxide (1M in 50% ethanol). The alkaline phase was acidified, the phenolic compounds reextracted in hexane:-diethyl ether, the solvent evaporated, and the residue dissolved in hexane before treatment with diazomethane. The coextracted lipids were removed by treatment with concentrated sulfuric acid (500 μl), and methoxylated metabolites were reconstituted in hexane. Finally, the samples were purified on an silica gel 60 column (500 mg; 35–70 μm; E. Merck, Darmstadt, Germany; washed with 30 ml hexane before use) using hexane, 2, 4, 6, 8, and 10% toluene in hexane as elution solvents (4 ml each). The eluted fractions were analyzed by gas chromatography–electron impact mass spectrometry (GC/MS) performed on a VG Auto Spec mass spectrometer operating at 70 eV, using a Model 5890 GC (Hewlett-Packard, Palo Alto, CA) with a 15 m DB-5ms column (J&W Scientific, Folsom, CA), using helium as a carrier gas (35–40 cm/s), run from 70 to 310°C at 10°C/min. Radioactive components detected in the nonextractable, water-soluble, and LFs were considered to be metabolites of PBDEs. Metabolites and parent BDEs were both detected and separately quantified by TLC in the unbound MFs. Only parent was detected in the unbound feces fractions.

Tissue metabolites. Tissue metabolites were not assessed in this study as previous studies have demonstrated that BDE in tissues from toxicokinetic studies are consistently parent compound. Orn and Klasson-Wehler (1998) demonstrated that parent BDE-47 was the only species found in mouse adipose, lung, kidney, and brain, while only trace amounts of metabolites were found in the liver. Hakk *et al.* (2002) demonstrated that no metabolites were observed in adipose, kidney, and lung extracts following administration of BDE-99. As with BDE-47, only trace amounts of metabolites were found in the liver. Similar results were observed with BDE-100 (Hakk *et al.*, 2006); no detectable metabolites were observed in liver, gastrointestinal tract, and adipose. The results of previous studies therefore suggest that the majority of radioactive BDE-47, -99, and -100 measured in the tissues evaluated in this study are likely to be parent compound. Because studies on BDE-153 were not available, the authors cannot discount that radioactive compound found in tissues may be metabolite and not parent compound.

Data analysis. For calculation of percent total dose, blood, fat, skin, and muscle mass were assumed to be 8, 8, 12, and 25%, respectively (Diliberto *et al.*, 2001). Percent dose data were normalized to recovery (60–90%) for comparison between congeners. An analysis of variance was used to compare exposure groups followed by Bonferroni posttests. Differences between treatment groups were considered significant when $p < 0.05$. All data are presented as mean ± standard deviation.

RESULTS

The toxicokinetics of four PBDE congeners were compared in female mice. In order to eliminate issues of differential absorption, BDEs 47, 99, 100, and 153 (Fig. 1) were administered intravenously via the tail vein. Tissue distribution was assessed 5 days following this single exposure. Excretion was

monitored daily; fecal metabolites were identified and urinary protein binding was assessed for each congener.

Tissue Distribution

Tissue concentrations are presented in four metrics (percent of dose, ng/g wet weight, percent dose/g tissue, and nmol/g tissue) for comparison (Table 1); however, most of the analyses in this manuscript are based on percent of dose and ng/g wet weight. All BDEs were found in the highest concentrations in fat, muscle, skin, and liver. Lungs, kidney, blood, and brain all had < 0.5% of the dose remaining in the tissue 5 days following exposure. Five days following exposure, 25.9 ± 11.3, 39.3 ± 6.8, 55.1 ± 4.7, and 74.9 ± 8.7% of the dose of BDE-47, -99, -100, and -153, respectively, remained in the tissues reported in Table 1.

A congener-dependent trend was apparent when the four congeners were compared on a wet weight concentration basis in all tissues except the liver and blood; concentrations were generally lowest for BDE-47, followed by 99, then by 100 and 153 which were often similar in concentration. Concentrations of BDE in the blood remain relatively constant regardless of the congener. Hepatic concentrations of BDE were also similar between congeners, with the exception of BDE-47, which was higher than the other congeners 5 days following exposure. This trend is highlighted by a liver to blood ratio for BDE-47 which is twice that of the other congeners (calculated from data in Table 1). The congener-dependent disposition pattern in the brain was also of interest (Fig. 2). Concentrations of BDE-153 in the brain were approximately 10 times higher than concentrations of BDE-47, whereas the BDE-153 concentration was only one to three times higher than BDE-47 for all other tissues when these two congeners were compared.

Excretion

Urinary excretion patterns were inversely related to disposition patterns; congeners with the lowest tissue concentrations

TABLE 1
Disposition of BDEs 47, 99, 100, and 153, 5 Days following a Single, Intravenous Administration (1 mg/kg)

Tissue	47	99	100	153
Fat	16.64 ± 8.58	24.69 ± 4.74	35.06 ± 2.40	44.00 ± 9.05
	(1432.36 ± 714.32)	(2142.95 ± 799.7)	(3906.8 ± 787.30)	(3082.97 ± 968.0)
	[10.76 ± 5.61]	[15.7 ± 2.61]	[22.59 ± 1.24]	[26.54 ± 5.58]
	{2.95 ± 1.47}	{3.79 ± 1.42}	{6.91 ± 1.39}	{4.78 ± 1.5}
Skin	3.39 ± 0.81	4.90 ± 1.08	6.82 ± 1.25	9.83 ± 1.24
	(19.33 ± 3.63)	(27.37 ± 6.18)	(50.01 ± 9.14)	(45.28 ± 7.44)
	[1.46 ± 0.38]	[2.08 ± 0.42]	[2.93 ± 0.49]	[3.95 ± 0.49]
	{0.04 ± 0.01}	{0.05 ± 0.01}	{0.09 ± 0.02}	{0.07 ± 0.01}
Muscle	3.80 ± 2.19	7.53 ± 3.09	10.92 ± 2.84	17.62 ± 3.84
	(10.25 ± 5.20)	(20.73 ± 11.98)	(39.02 ± 13.51)	(38.43 ± 6.95)
	[0.79 ± 0.47]	[1.53 ± 0.61]	[2.26 ± 0.62]	[1.75 ± 0.2]
	{0.02 ± 0.01}	{0.04 ± 0.02}	{0.07 ± 0.02}	{0.06 ± 0.01}
Liver	1.71 ± 0.41	1.63 ± 0.27	1.73 ± 0.25	2.24 ± 0.27
	(44.49 ± 31.83)	(22.70 ± 4.73)	(31.81 ± 2.92)	(25.21 ± 4.17)
	[2.19 ± 0.69]	[1.73 ± 0.35]	[1.89 ± 0.34]	[2.16 ± 0.22]
	{0.09 ± 0.07}	{0.04 ± 0.01}	{0.06 ± 0.01}	{0.04 ± 0.01}
Lung	0.07 ± 0.01	0.20 ± 0.03	0.13 ± 0.06	0.37 ± 0.15
	(10.01 ± 1.84)	(24.23 ± 6.49)	(22.73 ± 4.35)	(38.01 ± 9.52)
	[0.76 ± 0.2]	[1.8 ± 0.25]	[1.37 ± 0.43]	[3.37 ± 1.05]
	{0.02 ± .004}	{0.04 ± 0.01}	{0.04 ± 0.01}	{0.06 ± 0.01}
Kidney	0.07 ± 0.02	0.09 ± 0.02	0.11 ± 0.03	0.21 ± 0.04
	(3.98 ± 0.81)	(4.36 ± 0.55)	(8.96 ± 1.09)	(9.31 ± 2.28)
	[0.3 ± 0.08]	[0.34 ± 0.08]	[0.53 ± 0.09]	[0.81 ± 0.17]
	{0.01 ± 0.002}	{0.01 ± 0.001}	{0.02 ± 0.002}	{0.01 ± 0.004}
Blood	0.20 ± 0.11	0.19 ± 0.03	0.22 ± 0.10	0.31 ± 0.05
	(17.14 ± 8.34)	(16.15 ± 2.18)	(23.15 ± 8.04)	(21.69 ± 4.37)
	[0.13 ± 0.07]	[0.12 ± 0.02]	[0.14 ± 0.06]	[0.19 ± 0.03]
	{0.04 ± 0.02}	{0.03 ± 0.004}	{0.04 ± 0.01}	{0.03 ± 0.01}
Brain	0.03 ± 0.01	0.05 ± 0.01	0.12 ± 0.02	0.34 ± 0.04
	(8.91 ± 1.79)	(17.73 ± 4.14)	(53.68 ± 8.12)	(96.71 ± 18.39)
	[0.07 ± 0.01]	[0.13 ± 0.02]	[0.31 ± 0.04]	[3.95 ± 0.49]
	{0.02 ± 0.004}	{0.03 ± 0.01}	{0.10 ± 0.01}	{0.15 ± 0.03}

Note. Percent dose, (ng/g wet weight), [%dose/gram tissue], and {nmol/gram tissue} presented as mean ± standard deviation.

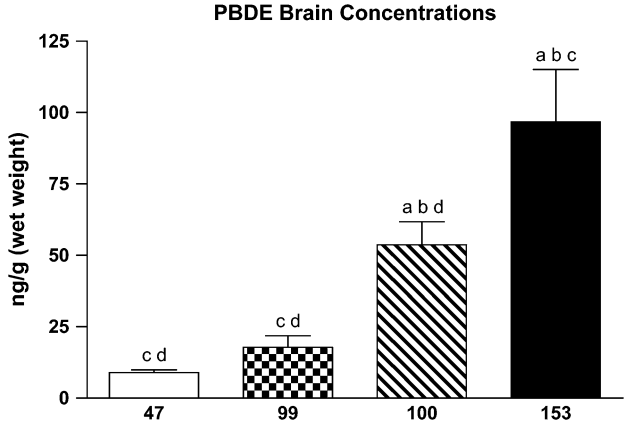


FIG. 2. Wet weight concentrations of PBDE in brain tissue 5 days following a single, iv administration (1 mg/kg). Statistical significance ($p < 0.05$) as compared to (a) BDE 47, (b) BDE 99, (c) BDE 100, and (d) BDE 153.

had the highest urinary concentrations. BDE-47 was most rapidly excreted in the urine, followed by BDEs 99, 100, and 153 (Fig. 3). Approximately 17, 8, 2, and 1%, respectively, were excreted in the urine within 24 h of an iv administration. Cumulatively, 40, 16, 6, and 2% were excreted in the urine at 5 days demonstrating major congener-dependent differences in urinary excretion. Fecal excretion followed a different pattern for the three lower brominated congeners (Fig. 4). A total of 16, 21, and 13% of BDEs 47, 99, and 100, respectively, were excreted on the first day. After 5 days, 32, 42, and 34% had been excreted, respectively. Fecal excretion of BDE-153 was the lowest of the four congeners studied: only 12% had been excreted within the first 24 h, increasing to 18% at 5 days.

Urinary Binding

Urine was analyzed for either protein binding by GPC analysis with a Sephadex G-75 column if sufficient radiochemical was present, or extracted and analyzed by TLC when insufficient amounts of radiochemical were available to perform G-75 binding studies. Analyses of BDE-47 and -99 urine excreted on day 1 revealed that the majority of BDE in the urine is protein-bound (98.6 and 59.6%, respectively). Pooled urine samples (days 1–5) were analyzed for protein binding for BDEs 100 and 153, and 95.9 and 55.1%, respectively, were also bound. SDS-PAGE analyses revealed that all BDE congeners bound covalently to an 18-kDa protein (data not shown). The binding protein has been identified as mouse major urinary protein (mMUP), which belongs to the same superfamily of proteins which include FABP and alpha-2μ, both of which are known to bind polyhalogenated aromatic hydrocarbons (Hakk *et al.*, 2002; Larsen *et al.*, 1990a,b, 1991). Three MUP isoforms exist in mice, all of which have the same molecular weight; however, the number of charged amino acids differ between the isoforms. BDEs 47 and 99 bind to MUP-1, whereas BDEs 100 and 153 appear to bind to MUP-2 and -3.

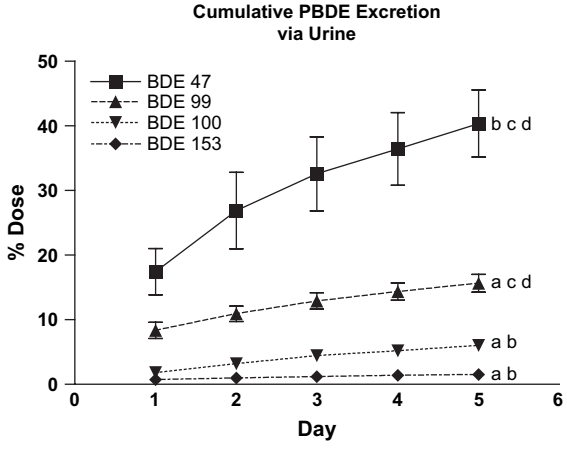


FIG. 3. Cumulative urinary excretion of PBDE (% dose) 5 days following a single, iv administration (1 mg/kg). Statistical significance ($p < 0.05$) as compared to (a) BDE 47, (b) BDE 99, (c) BDE 100, and (d) BDE 153 based on cumulative percent dose excreted (daily significance not shown).

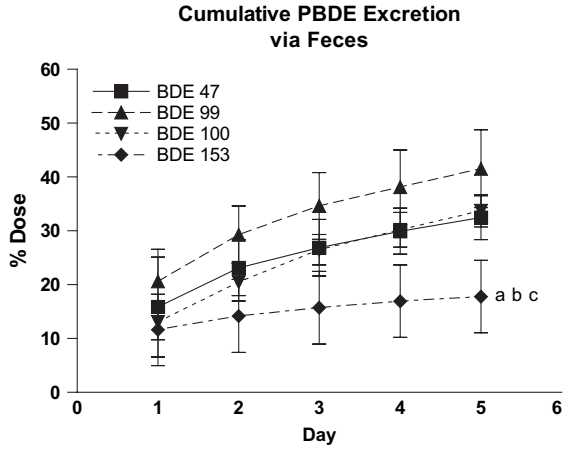


FIG. 4. Cumulative fecal excretion of PBDE (% dose) 5 days following a single, iv administration (1 mg/kg). Statistical significance ($p < 0.05$) as compared to (a) BDE 47, (b) BDE 99, (c) BDE 100, and (d) BDE 153 based on cumulative percent dose excreted (daily significance not shown).

Metabolism

Analysis of MFs in feces is presented in Table 2. The percent of dose excreted per day is provided in the first column of the table. Complete metabolite analysis is provided in the remaining columns, which refer to the percent of the daily BDE excreted corresponding to each MF. Total metabolism in feces is the sum of the radioactivity bound to lipid, soluble in water, nonextractable from feces, and a portion of the extractable feces determined by TLC. For instance, approximately 16% of the intravenously administered dose of BDE-47 was excreted in the feces on day 1, of which 46% was metabolite and 54% was parent. While the percent of parent decreased to 30% by day 5, only 2.5% of the administered dose was excreted in feces on day 5.

All fecal metabolites were characterized as their respective methoxylated derivatives. BDE-99 was the most readily metabolized congener; almost 80% of the fecally derived

TABLE 2
Metabolite Analysis of Daily BDE Fecal Extracts following a Single Intravenous Dose in Female Mice

		Metabolite analysis of percent daily excreted dose					
		Metabolite breakdown					
Congener, day	Percent daily excreted dose	Nonextractable	Water soluble	Lipid bound	Unbound metab.	Total metabolite	Parent
BDE 47							
Day 1	15.86 ± 9.26	14.87	14.24	5.38	11.67	46.16	53.84
Day 2	7.28 ± 4.28	18.89	17.81	7.03	32.31	76.04	23.96
Day 3	3.75 ± 0.40	24.78	20.82	2.3	13.86	61.76	38.24
Day 4	3.06 ± 1.38	34.99	7.45	4.06	10.72	57.22	42.79
Day 5	2.51 ± 0.42	10.85	50.03	1.57	7.46	69.91	30.09
BDE 99							
Day 1	20.64 ± 5.95	27.14	19.22	10.86	21.61	78.83	21.18
Day 2	8.64 ± 1.74	28.18	19.31	10.49	31.87	89.85	10.15
Day 3	5.33 ± 1.24	26.27	20	9.1	31.22	86.59	13.4
Day 4	3.50 ± 1.25	22.21	24.07	8.36	27.08	81.72	18.28
Day 5	3.42 ± 0.64	21.95	25.14	9.09	31.17	87.35	12.65
BDE 100							
Day 1	13.09 ± 3.33	21.85	27.76	13.54	17.89	81.04	18.96
Day 2	7.39 ± 1.27	28.55	12.75	8.93	35.78	86.01	13.98
Day 3	6.01 ± 2.13	0	35.68	18.59	32.34	86.61	13.39
Day 4	3.71 ± 0.80	30.84	41.38	7.95	4.52	84.69	15.32
Day 5	3.52 ± 0.53	17.54	22.3	10.22	23.6	73.66	26.34
BDE 153							
Day 1	11.60 ± 2.59	12.49	17.24	9.44	45.27	84.44	15.55
Day 2	6.61 ± 0.46	9.55	13.84	5.42	32.21	61.02	38.98
Day 3	1.54 ± 0.22	14.04	27.17	2.41	31.17	74.79	25.21
Day 4	1.22 ± 0.17	7.6	48.74	1.24	5.58	63.16	36.84
Day 5	0.85 ± 0.15	7.56	38.78	2.59	17.48	66.41	33.6

radioactivity was identified as metabolites within the first 24 h (calculated as the sum of metabolites detected in nonextractable, extractable, water-soluble and LFs, Table 2). At day 5, almost 90% of BDE-99 excreted in the feces was metabolite. MS of the methoxylated metabolites present in the extractable portion of the feces revealed that oxidation and oxidation/debromination were common metabolic pathways for each of the PBDE congeners (Table 3). A series of three monohydroxylated isomers of BDE-47 were identified as metabolites in the extractable portion of feces (Table 3). BDE-99 metabolites identified were four monohydroxylated isomers, two monohydroxylated/debrominated isomers, and a single dihydroxylated metabolite. BDE-100 metabolites characterized included three monohydroxylated isomers, monohydroxylated/debrominated isomers, and a single dihydroxylated/debrominated metabolite (Table 3). BDE-153 did not show an increasing level of metabolite formation over time. BDE-153 metabolites identified included three monohydroxy isomers, two monohydroxy/debromo isomers, and one monohydroxy/di-debromo isomer. A weak mass spectral molecular ion signal at M+ 684 (6Br) was indicative of a trace amount monothiomethyl BDE-153. It is of particular interest to note that simple debromination did not occur *in vivo* for any of the congeners in this study.

A cumulative comparison of parent and MFs is provided in Figure 5; clearly, BDE-47 is excreted as parent compound in both urine and feces to a much greater extent than the other congeners in this study. In contrast, metabolites dominated urinary and fecal-derived radioactivity for BDEs 99 and 100, and BDE-153. Furthermore, cumulative urinary excretion of BDE-47 was greater than fecal excretion, which is contrary to the other congeners in this study.

DISCUSSION

We have compared tissue distribution and elimination of BDE congeners 47, 99, 100, and 153 in mice following a single, intravenous dose (1 mg/kg). Previous studies have individually reported on the ADME of BDEs 47, 99, and 100 following oral exposure in rats or mice; however, no studies have compared the congeners in a single study. The results of this study are generally in agreement with the results of previous BDE-47 and BDE-99 toxicokinetic studies in mice (Chen *et al.*, 2006; Orn and Klasson-Wehler, 1998; Sanders *et al.*, 2006b; Staskal *et al.*, 2005, 2006b). Peer-reviewed studies on the toxicokinetics of BDEs 100 and 153 in mice were not available at the time this study was conducted.

TABLE 3
Mass Spectra of Methyl Ether Derivatives of Compounds Extracted from Feces of Female Mice Administered BDE-47 (1.0 mg/kg), BDE-99 (2.1 mg/kg), BDE-100 (1.9 mg/kg), and BDE-153 (1.8 mg/kg) as a Single Intravenous Dose

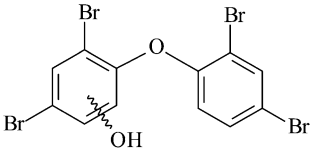
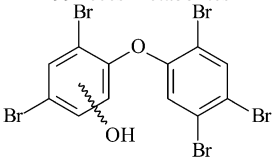
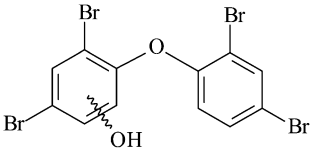
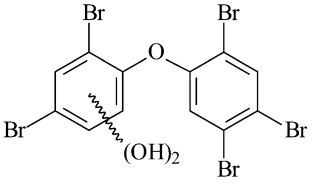
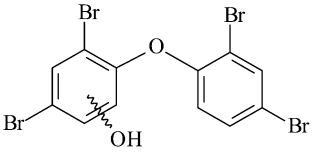
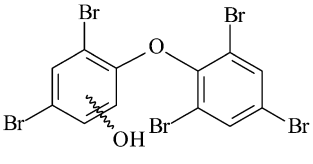
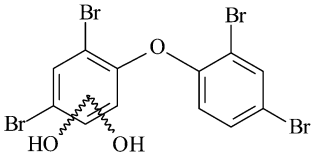
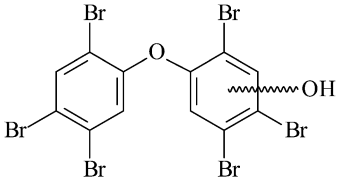
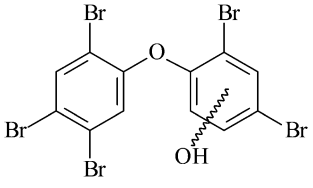
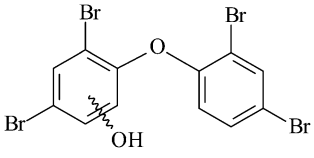
BDE-47 feces metabolites	Methylated mass spectral fragments
	M^+. 512 (4Br) M-15 (497; 4Br) M-158 (354; 2Br) M-CH_3Br_2 (339, 2Br) M-201 (311; 2Br)
OH-TBDE *(Three isomers)*	
BDE-99 feces metabolites	
	M+ 590, 5Br M-15 (575, 5Br) M-94 (496, 4Br) M-2Br (432, 3Br) M-CH_3Br_2 (419, 3Br) M-$COCH_3Br_2$ (389, 3Br)
OH-PeBDE *(Four isomers)*	
	M^+. 512 (4Br) M-15 (497; 4Br) M-94 (418; 3 Br) M-158 (354; 2Br) M-CH_3Br_2 (339, 2Br) M-201 (311; 2Br)
OH-TBDE *(Two isomers)*	
	M^+. 620 (5Br) M-15 (605; 5Br)
diOH-PeBDE *(One isomer)*	
BDE-100 feces metabolites	
	M^+. 512 (4Br) M-15 (497; 4Br) M-94 (418; 3 Br) M-158 (354; 2 Br)
OH-TBDE *(Three isomers)*	
	M^+. 590 (5 Br) M-15 (575) M-158 (432, 3Br) M-CH_3Br_2 (417; 3Br) M-$COCH_3Br_2$ (389, 3Br)
OH-PeBDE *(Three isomers)*	

TABLE 3—*Continued*

	M^+. 542 (4 Br) M-2Br (384, 2Br)
Di-OH-TBDE *(One isomer detected)*	
BDE-153 feces metabolites	
	M^+. 668 (6 Br) M-94 (574, 5Br) M-2Br (510, 4Br) M-$COCH_2Br_2$ (467, 4Br)
OH-HxBDE *(Three isomers)*	
	M^+. 590 (5 Br) M-15 (575) M-94 (496, 4Br) M-158 (432, 3Br) M-CH_3Br_2 (417; 3Br) M-$COCH_3Br_2$ (389, 3Br)
OH-PeBDE *(Two isomers)*	
	M^+. 512 (4 Br)
OH-TBDE *(One isomer)*	

All of the congeners studied distributed to lipophilic tissues. Generally, tissue concentrations of BDE-47 were the lowest, followed by BDEs 99 and 100. BDE-153 tissue concentrations were highest 5 days following exposure, which was likely due to differential excretion between congeners. When tissue concentrations (%dose/g) were normalized to the percent dose remaining in the body on day 5, the impact of differential excretion on tissue concentrations was virtually eliminated: little to no differences in concentration in fat, skin, muscle, lung, or kidney were observed among the different congeners (calculated from data in Tables 1 and 2 and Fig. 3).

However, even when tissue concentrations were normalized to percent remaining in they body, the concentrations of BDE-153 in the brain were much higher than other congeners tested, i.e., 0.3, 0.3, and 0.5% dose/g for BDEs 47, 99, and 100, respectively, versus 6.6% dose/g for BDE-153. At 5 days following exposure, wet weight concentrations of BDE-153 in

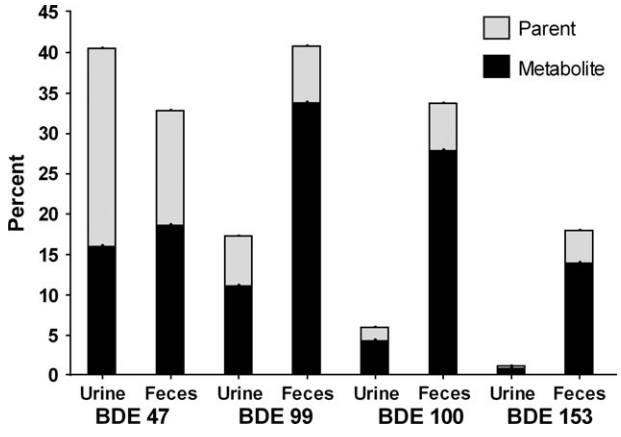


FIG. 5. Comparison of cumulative percent dose excreted as parent or metabolite for each congener in urine and feces from female mice. Parent: metabolite ratios for urine were 1.5, 0.5, 0.4, and 0.2 for BDEs 47, 99, 100, and 153, respectively. Ratios in feces were 0.7, 0.2, 0.2, and 0.3, respectively.

the brain were approximately four times higher than what was found in the blood. This was in contrast to brain to blood ratios of 0.5, 1.2, and 2.3 for BDEs 47, 99, and 100, respectively. Higher concentrations of BDE-153 in the brain could potentially be attributed to differences in blood carrier protein systems, the inherently slower movement of higher brominated compounds into fat, similar to what has been observed in PCBs (King *et al.*, 1983; Lutz *et al.*, 1977; Tuey and Matthews, 1980a,b), or perhaps to an increased ability of BDE-153 to penetrate the blood:brain barrier due to differential interaction with active transport proteins.

BDEs 99 and 100 were the only two congeners administered on an exactly equimolar basis (1.9 μmol/kg, although the other congeners were very similar at 2.1 and 1.8 for BDEs 47 and 153, respectively). However, the distribution and elimination for these two pentaBDEs were distinctly different. While the patterns of distribution were similar, tissue concentrations of BDE-100 were higher than BDE-99. Again, this was likely due to differences in excretion as tissue concentration differences were generally eliminated when normalized to %dose remaining in the body at 5 days. More BDE-99 was eliminated in both feces and urine when compared to BDE-100, which was partly due to greater metabolism of BDE-99 than 100. The reason for the differential metabolic capacities could be explained by differential enzyme induction, bioavailability, and/or stereochemistry. Results presented by Chen *et al.* (2006) demonstrated that BDE-99 has the potential to induce its own metabolism following repeated exposures in rats, and that it undergoes more extensive metabolism than BDE-47.

Identification of various oxidative metabolites suggests that cytochrome P450s are likely involved in PBDE metabolism. For each congener, multiple monohydroxylated isomers were observed (Table 3). Only BDEs 99, 100, and 153 displayed both monohydroxylated/monodebrominated metabolites, while BDE-153 underwent monohydroxylation/di-debromination (Table 3). Both penta PBDE congeners, i.e., BDE-99 and -100, were observed to undergo dihydroxylation, however, no accompanying debromination was observed with BDE-99, while with BDE-100 concomitant di-debromination occurred. Although only a minor metabolic product in previous studies in rat with BDE-47 (Orn and Klasson-Wehler, 1998) and BDE-99 (Hakk *et al.*, 2002), and in the mice BDE-47 and -99 studies by Sanders *et al.* (2006) and Chen *et al.* (2006), respectively, no thiols were detected for BDEs 47, 99, or 100 in the present mouse study but a trace amount was detected in the BDE-153 feces. The formation of thiol metabolites is an indication that the mercapturic acid pathway can be utilized for PBDE metabolism.

Analyses of the results in this study reveal that metabolism alone does not explain the differences in excretion patterns between PBDE congeners. The amount of BDE-47–derived radioactivity excreted in the urine was over twice that of BDE-99, seven times that of BDE-100, and almost 40 times that of BDE-153; however, analyses of the urine revealed that > 98% of BDE-47 in day 1 urine was parent compound. Orn and Klasson-Wehler (1998) observed similar results in mice as 33% was excreted in 5 days and only parent compound was identified. Orn and Klasson-Wehler (1998) also observed a major species difference in urinary excretion as rats in the same study excreted less than 0.5% within 5 days of exposure. Furthermore, Sanders *et al.* (2006) reported that male mice excreted up to 100-fold more BDE-47 in urine than did male rats following a single administration.

While not as pronounced as BDE-47, BDE-99 has also demonstrated marked species difference in urinary excretion. Hakk *et al.* (2002) reported that rats excreted approximately 23% of a single oral dose of BDE-99 within the first 24 h, but less than 0.5% was excreted into the urine. In contrast to rats, mice in the present study excreted ~ 30% of the intravenously administered BDE-99 into urine and feces within 24 h of exposure; approximately one-third of which was in the urine. Chen *et al.* (2006) also found similar distinctions between species and also identified major sex-dependent urinary excretion differences for BDE-99. Male rats and mice given a single iv dose excreted 2.7 and 19.9%, respectively, into the urine within 24 h following exposure; oral exposure resulted in 1.6 and 7.8% in the urine, respectively.

Urinary excretion appears to drive the major differences in elimination observed in multiple studies between individual congeners, animal sex, as well as between species (Chen *et al.*, 2006; Orn and Klasson-Wehler, 1998; Sanders *et al.*, 2006; Staskal *et al.*, 2005). The results of the present study may offer support for the observed sex, congener, and species differences in urinary excretion: all the BDEs investigated, especially BDE-47, bind to MUP in mice. The majority of MUP is synthesized in the liver, secreted in the serum, circulated at low levels, and rapidly filtered by the kidney and excreted into the urine. Because adult male mice secrete up to 20 times more MUP from the liver than females, this could explain the differential observations made by Sanders *et al.* (2006) and Staskal *et al.* (2006b) in which significant increases in the

percent of BDE-47 excreted following repeated exposures were observed in male mice but not in females. A rough calculation of BDE-47 urinary concentration at day 1 (assuming 5 ml urine volume, MUP concentration of 5 mg/ml (280μM), and resulting urinary BDE-47 concentration of 3.2μM) demonstrates that saturation of MUP by urinary PBDEs is unlikely, even though saturation of urinary excretion of BDE-47 was observed by Staskal *et al.* (2005).

MUP is also under developmental and hormonal control, which may explain the age-dependent excretion of BDE-47 reported by Staskal *et al.* (2006a). Mice dosed with BDE-47 on postnatal days 10, 22, 28, and 40 demonstrated that urinary excretion increased with age. Furthermore, MUP mRNA expression in the liver is regulated by thyroxine, peptide growth hormones, and androgens (particularly testosterone). It is also interesting to note that Sanders *et al.* (2006) observed differential distribution of BDE-47 to the thyroid and thymus in male mice following repeated exposure. This connection may be of particular interest when evaluating mechanisms of toxicity for PBDEs, considering previous demonstrations of PBDE-induced endocrine disruption, including anti-androgenic activity, as well as impacts on thyroid hormones. The SDS-PAGE results of this study also demonstrated that BDEs 99, 100, and 153 bind to MUP. Further studies in our laboratory will characterize MUP binding in mouse urine. However, active transporters may still be involved in PBDE distribution (i.e., brain) and excretion in mice, particularly in the early life stages when maximal MUP production has not yet been achieved.

In conclusion, the results of this study demonstrated that bromination pattern plays a role in the distribution, metabolism, and excretion of PBDEs in mice. BDE-47 usually dominates the PBDE congener profiles found in human and wildlife samples in the United States and worldwide; however, increasing levels of BDEs 99, 100, and 153 have recently been reported. The congeners evaluated in this study have previously demonstrated the potential for developmental neurotoxicity, developmental reproductive toxicity, and endocrine disruption in rodent studies. Because the mechanism(s) of toxicity are not understood, it is important to consider the differential toxicokinetic parameters associated with each congener when assessing the risk to human health from individual PBDE congeners as well as the commercial PBDE mixtures.

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